

ABSTRACT OF THE DISCLOSURE

5 Methods and materials are provided for stably
introducing any gene into a specific locus in the genome of a
microorganism such as yeast without the addition of any drug
resistance genes. Specifically provided herein are new
genetically engineered inositol-overproducing Saccharomyces
10 cerevisiae strains obtained by using a novel set of yeast
integration plasmids that allow the safe, stable, and controlled
introduction of homologous as well as heterologous genes into
the host genome. In particular, specific loci of the S.
15 cerevisiae yeast genome can be targeted with single or multiple
copies of a specific gene that is desired to be expressed or a
given set of specific genes that the host can use without the
addition of any drug resistance genes. The principles of this
new methodology can also be used for the construction of other
recombinant yeast and bacterial strains as well as higher
eukaryotic cells.